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Isolation of Nematophagous Fungi against Root-knot Nematode and Their Growth *in Vitro*

뿌리혹 線蟲에 대한 寄生 天敵 眞菌의 分離 및 이들의 生長에 미치는 環境條件

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ABSTRACT Nematophagous fungi were successfully isolated by baited plating, centrifugation technique of soil, and direct isolation from naturally infested nematodes. Predominant seven fungi isolated were identified as *Arthrobotrys arthrobotryoides, A. conoides, A. oligospora, Dactylella lobata, Fusarium oxysporum, Monacrosporium ellipsosporum* and *Harposporium anguillulae*. Of these, six fungi were tested for cultural characteristics except *H. anguillulae*, extremely fastidious fungus in artificial media. Among 14 media tested in this experiment, Cornmeal Agar (CMA) and Oatmeal Agar (OMA) were the most suitable media for growing all six nematophagous fungi. Weakly saprophytic *M. ellipsosporum* also grew vigorously on these two media. The radial growth, dry weight and sporulation of the fungi tested were quite diverse depending on the culture media tested. *D. lobata* revealed good growth and abundantly sporulated on Glucose Peptone Agar (GPA). Although over-all growth of *F. oxysporum* was not satisfactory on Sucrose Nitrate Agar (SNA), the sporulation was best on this medium. Optimum conditions for mycelial growth and sporulation of nematophagous fungi ranged pH 5—8 and 20—30°C on SNA. *D. lobata* and *F. oxysporum* grew vigorously and most profusely sporulated on all media tested. They turned out as most promising biocontrol agents for their aggressive growth and sporulation over the ranges of temperature and pH ranges.

KEY WORDS nematophagous fungi, isolation, growth and sporulation

抄 錄 晋州 近郊와 琴山, 鳴石 等의 線蟲發生 常習地의 健全 植物의 根圈土壤으로부터 미끼 利用法, 遠心分離法으로 天敵眞菌을 分離하였고, 또 自然感染線蟲으로부터 直接 天敵 眞菌을 分離하여, 線蟲에 대한 寄生能力이 優秀한 다음 7菌株를 同定한 結果 *Arthrobotrys arthrobotryoides, A. conoides, A. oligospora, Dactylella lobata, Fusarium oxysporum, Monacrosporium ellipsosporum*과 *Harposporium anguillulae*였다. 그중 人工培養基에서 增殖이 어려운 *H. anguillulae*를 除外한 6菌株의 培養上의 特徵을 調査하였던 結果를 要約하면 다음과 같다. 14種類의 培養基에서 乾物重과 菌叢生長 및 胞子形成量을 調査 했던 바, CMA와 OMA에서는 供試된 6菌株의 生長狀態가 良好하며, 腐生性이 매우 약하여 一般培養基上에서는 生長이 극히 不良한 *M. ellipsosporum*도 다른 培養基에서보다 뛰어나, 培養上의 特徵을 調査하기에 充分하였다. 菌株에 따라 乾物重, 菌叢生長 및 胞子形成에 큰 差異를 나타내어 多樣하였다. *D. lobata*는 GPA에서 菌糸生長과 胞子形成이 특히 優秀하였고, *F. oxysporum*은 SNA에서 胞子形成은 가장 優秀하였으나 菌糸生長은 比較的 良好한 편이었고, CM에서는 胞子形成은 良好하였고, 菌糸生長은 最適이었다. SNA上에서 線蟲寄生菌의 乾物重, 菌叢生長 및 胞子形成의 最適條件은 pH5—8 및 20—30°C였다. 이들중 *D. lobata*와 *F. oxysporum*의 胞子形成 및 菌糸生長이 다른 菌에 비해 越等히 優秀하였다.

檢 索 語 線蟲寄生天敵眞菌, 分離, 菌糸生長과 胞子形成

Plant parasitic nematodes occur naturally in undisturbed soils. Establishment of an agroecosystem promotes the rapid increase of endemic pathogenic species and allows introduction of nonendemic species that quickly establish dominance(Cho 1985, Good 1972). Nematodes of 16 genera were reported to associate with 18 horticultural crops throughout Korea(Cho 1985). Rootknot nematodes

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are most predominant and economically important parasites causing crop losses in Korea (Cho 1985, Choi 1979, Choi & Choo 1978, Choo 1978) and elsewhere(DiVito & Carella 1986). In addition to direct to damage to crops, *Meloidogyne* infected plants are predisposed to be fungal and bacterial diseases (Ali, Trabulst & Abd-Elsamen 1981, Caperton, Martyn & Starr 1986, Golden & Van Gundy 1975, Morrell & Bloom 1981, Sidhu & Webster 1977, Weltry, Barker & Lindsey 1980). Thus, control of plant parasitic nematodes would help reduce the damage by either fungal or bacterial plant diseases.

Control of nematode by crop rotation has been suggested(Dickerson, Franz & Lash 1978, Kerry 1984). The use of chemical pesticides have been increased rapidly, however, pesticides were a short-term solution to the problem of nematodes, as the nematode populations increased several months after the nematicides use or new races of nematodes developed due to selection pressure caused by the use of resistant varieties. In nature, fungi continously destroy nematodes in virtually all soils. Microbial and fungal control of nematode are very important, biologically and in some instances economically. The fungal antagonists of nematodes consist of a great variety of organisms belonging to widely divergent orders and families of fungi(Harrd 1986, Mankau 1980a, Mankau 1980b). Unfortunately, however, no trials on biocontrol of any nematodes has been reported so far in Korea. Such a situation prompted us to challenging work on biocontrol of root-knot nematodes. Therefore, this study was undertaken to isolate and identify the potential nematophagous fungi, and determine optimum condition for the mycelial growth and sporulation of the isolated antagonisms *in vitro*.

MATERIALS AND METHODS

Collecting soils and egg masses as a source of nematophagous fungi

Soil samples were collected from 4 different sites of Chin-ju and Chin-yang. The soil sampling sites selected were the rhizosphere at depths of 10—45cm where various plant

Table 1. Description of 67 isolates of nematophagous fungi

Scientific name of fungi	Method of isolation	No. of Isolates	Locality	Source
Arthrobotrys arthrobotryoides	BP[a]	5	Chinju(Chojeon)	Soil
		6	Chinyang(Geumsan)	Soil
	DI	3	Chinju(Chojeon)	Nematode
A. conoides	BP	3	Chinju(Chojeon)	Soil
		3	Chinju(Jangje)	Soil
		3	Chinyang(Geumsan)	Soil
A. oligospora	BP	2	Chinyang(Geumsan)	Soil
Dactylella lobata	BP	5	Chinju(Chojeon)	Soil
		8	Chinyang(Geumsan)	Soil
Fusarium oxysporum	DI	12	Chinyang(Geumsan)	Nematode
		2	Chinyang(Myeongseuk)	Nematode
	EM	7	Chinyang(Myeongseuk)	Egg mass
Monacrosporium ellipsosporum	DI	4	Chinyang(Myeongseuk)	Nematode
Harposporium anguillulae	DC	4	Chinyang(Geumsan)	Soil

[a] BP, Baited plating; DI, Direct isolation from naturally infested nematodes; EM, Isolation from egg masses of *Meloidogyne* sp.; DC, Differential centrifugation of soil suspension

were growing healthy under the plastic film houses and fields. Egg masses were collected from tomato plant at each site. The description and designation of the collected soils and egg masses were summarized in Table 1. The soil samples were stored in plastic pot which was planted in tomato or pepper placed in a greenhouse for later isolation of nematophagous fungi.

Isolation of nematophagous fungi

Baited plating

To isolate nematophagous fungi from field soil, nematode suspension was introduce to Water Agar (WA) or weak CMA(0.25 to 0.5 strength, Difco), which were previously sprinkled lightly with 0.5 to 1.0g of soil or organic debris. Five days after treatment, Petri plates were inspected microscopically to pick out the delicate conidiophore or hyphae growing from dead hosts. Plate were inspected at 1 to 3 day intervals for several weeks.

Differential centrifugation of soil suspension

About 200g well-mixed soil was added to 250ml water in a flask, and blended for 30 seconds by Tissue homogenizer (MSE Scientific Instrument, England). The soil water mixture was passed through a soil screen of 850μm (BS 410, Laboratory Test Sieve, Endecotts Ltd, London, England) to remove the coarse material. The mixture was passed through a further, finer of 250μm screen and the debris on the screen was discarded. The sample passing through the screen was centrifuged at 750g for 20minutes to remove the heavier soil particles and large spores of the predatory fungi. The supernatant was decanted, retained and centrifuged again at 2,500g for 20minutes. The pellet was saved and stirred with a glass rod by adding a few drops of water. The mixture(0.1ml) was spreaded on WA. Heavy suspension(1 ml) of nematodes were drop-wisely added to each plate and the plates were incubated for one week and inspected daily thereafter.

Detection from naturally infested nematodes

The fresh nematode suspensions obtained from Baermann funnel after 24 hours were centrifuged at 1,000g for 3 minutes. The supernatant was discarded and the nematodes resuspended in a further 10ml sterile distilled water and again centrifuged. This process was repeated four times. After the final wash, nematodes were transferred to plates of WA by adding 1 ml of the suspension and plates were incubated at 26°C for 3 days.

To facilitate the isolation of fungus-infected nematodes in soil, the nematodes in collecting tubes were allowed to stand at room temperature for 3 more days. Then, the fungus-infected or immobile nematodes were picked up easily by microscopic examination under low magnification.

Isolation from egg masses of *Meloidogyne* sp.

The gelatinous matrix of *Meloidogyne* sp. egg masses collected from host plants in the field was partially dissolved by treatment in 1%(w/v) NaOCl for about 2min and the eggs were examined for parasitic fungi. Clumps of parasitised eggs are washed in sterile water and transferred to WA.

Identification of nematophagous fungi

Isolates selected as nematophagous fungi were cultured on WA at 26°C incubator under 60watts cool white fluorescent light

illumination for 4 days. A round disk of agar medium, 4 mm in diameter, was transferred from agar plate to slide glass to examine microscopically conidiophore branching system, sterigmata, predaceous organ, chlamydospore etc., for identificaton of the fungi by the method of Haard(1986), Mitsui(1983), and Cook and Baker(1983).

Experiments on the laboratory conditions for growth and sporulation of nematophagous fungi

The fungi in these tests were *A. arthrobotryoides, A. conoides, A. oligospora, D. lobata, F. oxysporum* and *M. ellipsosporum.* The fungi were grown on CMA or Potato dextrose Agar (PDA) at 26°C incubator for 4days. The inoculum from either medium consisted of a 4 mm mycelial disc taken from near the edge of the colony. The growth of the fungi exposed to the different treatments was measured for dry weight and/or the radial growth of the mycelium and sporulation. First, broth media, 50ml of each, in 250 ml Erlenmeyer flasks were inoculated with either one of the fungi and still-cultured, with daily hand shaken, in 26°C incubator for 14days. Mycelial mats were vacuum filtered on filter paper(Whatman No. 3) and dried in oven at 90°C for 30minutes. Secondly, colonies developed on agar media were measured along the two axes at right angle the colony after 4days incubation at 26°C. Conidia were removed from the agar surface by pipetting 10ml of distilled water (0.02% Tween 80) onto the surface and gently rubbing the surface with a sterile brush and conidia were counted by a hemocytometer. Four replications were made in all experiments.

Effect of media

Mycelial growth and sporulation of the above mentioned nematophagous fungi were determined using the following agar media: Ashour's Nutrient Medium (ANM), Barnes' Agar(BA), CMA, Crabill's Media(CM), Czapek Solution Agar(CSA), GPA, Leonian's Agar (LA), Malt Extract Agar (MA), Nutrient Agar (NA), WA, OMA, PDA, SN A, and Yeast Extract Agar(YEA)(Jeong 1988, Reyes & Webster 1977). All media used were adjusted to pH6.5 and sterilized by autoclaving for 30min at 121°C.

Effect of temperature and pH

The seeded SNA plates were subjected to different temperature of 10, 15, 20, 25, and 30 °C. Also they were grown on SNA with pH ranging from 3.0 to 9.0 at interval of 1unit adjusted with photassium phosphate buffer.

RESULTS AND DISCUSSION

Identification of nematophagous fungi

Arthrobotrys Corda is a genus of Hyphomycetes, and some of which are apparently distributed in world-wide(Harrd 1986, Kerry 1984, Mankau 1980a, Mankau 1980b). The distinguishing characteristics was the formation of conidia on sterigmata in a whorled pattern at the tip and nodes of the conidiophore. The conidiophores in this genus are hyaline, septate, erect, single, free, and distinct. They may originate from either prostrate or aerial mycelium and may be branched or unbranched. Chlamydospores are found in some species, but occur only in old cultures. They are yellowish and spherical to oblong (Table 2).

The isolate A_3 was identified as *A. arthrobotryoides* with colony in pure culture white or pale rose, spreading rapidly, forming adhesive loops in pure culture, frequently networks. Conidiophore was highly branched and its tip irregularly swollen. Obovoid con-

idia were borne solitary on blunt wartlike sterigmata. Chlamydospores were not observed (Table 2).

Isolate A_4 was identified as *A. conoides* with colonies in pure culture white to yellow, spreading radially. Conidiophore was unbranched and its tip irregularly expanded. Conidia were elongate obovoid. Intercalary chlamydospores were in chains, round to oblong, and abundant in old cultures (Table 2).

Isolate A_5 was identified as *A. oligospora* with colonies in pure culture, white to yellow, spreading radially. Conidiophores were unbranched except in rare instances with nodular shape. This branching of conidiophore in rare cases is very important to differentiate this isolate from *A. oviformis*, which mostly have branched conidiophore. Chlamydospores were yellow, spherical or cylindrical, single, or in chains(Table 2).

The Hyphomycetes genus *Dactylella* was characterized by conidiophores erect, simple, hyaline, septate, on hyphae branched, bearing one or several spores acropetally. Conidia were ellipsoidal to fusoid or cylindrical, several to multiseptate, hyaline, tapering toward the ends, with one cell (usually the centermost cell wider and longer than the others). Isolate D1 was identified as *D. lobata* with conidiophores erect, septate, 250μm long, bearing single apical conidia. Hyaline 4-celled conidia, often germinated directly to form an adhesive lobe(Cook & Baker 1983) (Fig. 1).

The Hyphomycetes genus *Monacrosporium* was characterized by conidiophores tall, usually simple, hyaline, slender, conidia (aleuriospores) single, hyaline several-celled, usually fusoid with one cell (near middle) larger. Isolate M_1 was identified as *M. ellipsosporum* with septate and hyaline mycelium 2—6μm wide. Conidiophores were hyaline, erect, 150—300μm long, bearing single conidium. Conidia were pyriform hyaline, 5-celled, 24—65 μm long, 7.5—19μm wide (Fig. 1).

Fusarium sp. were isolated from profuse mycelial development egg mass on WA. The culture was exclusively produced microconidia at early stage of infection on nematodes. Micro-conidia abundant, mostly borne on short (often reduced), simple, lateral phialides /or from sparsely branched conodiophores. This is a very distinctive character of *F. oxysporum* along with exclusive microconidia production in broth culture which we have tried. Macro-conidia were pedicellate. Chlamydospores terminal or intercalary in hyphae, hyaline, rough walled. *F. oxysporum* has a world wide distribution, mostly as a soil saprophyte in a wide range of pathogencity to plants, in which specialized pathotypes may cause vascular wilt or damping off. The fungus identified in this experiment was tested for plant pathogenicity to radish, chinese cabbage, tomato, cucumber and pepper. It was nonpathogenic to these crops tested (Fig. 1). This fungus was given sperical attention because it is known as common facultative saprophytes in variety of soil environment. The endoparasitic forms persist in soil principally as conidia or sometimes as chlamydospores released into the soil from disintegrating nematodes. Generally conidia of endoparasitic species are relatively small or if long are very narrow; predaceous species on the other hand have large, bulky spores(Barron 1970). Isolate H_7 was identifieda s *H. anguillulae* with mycelium not extensive, bearing lateral, globose phialides single or in groups. Conidia (phialospores) were hyaline, 1-celled, elongated, curved to hooked, in moist clusters. This fungus is different from *H. helicoides*(Barron 1970) (Fig. 1).

From the above characterization procedu-

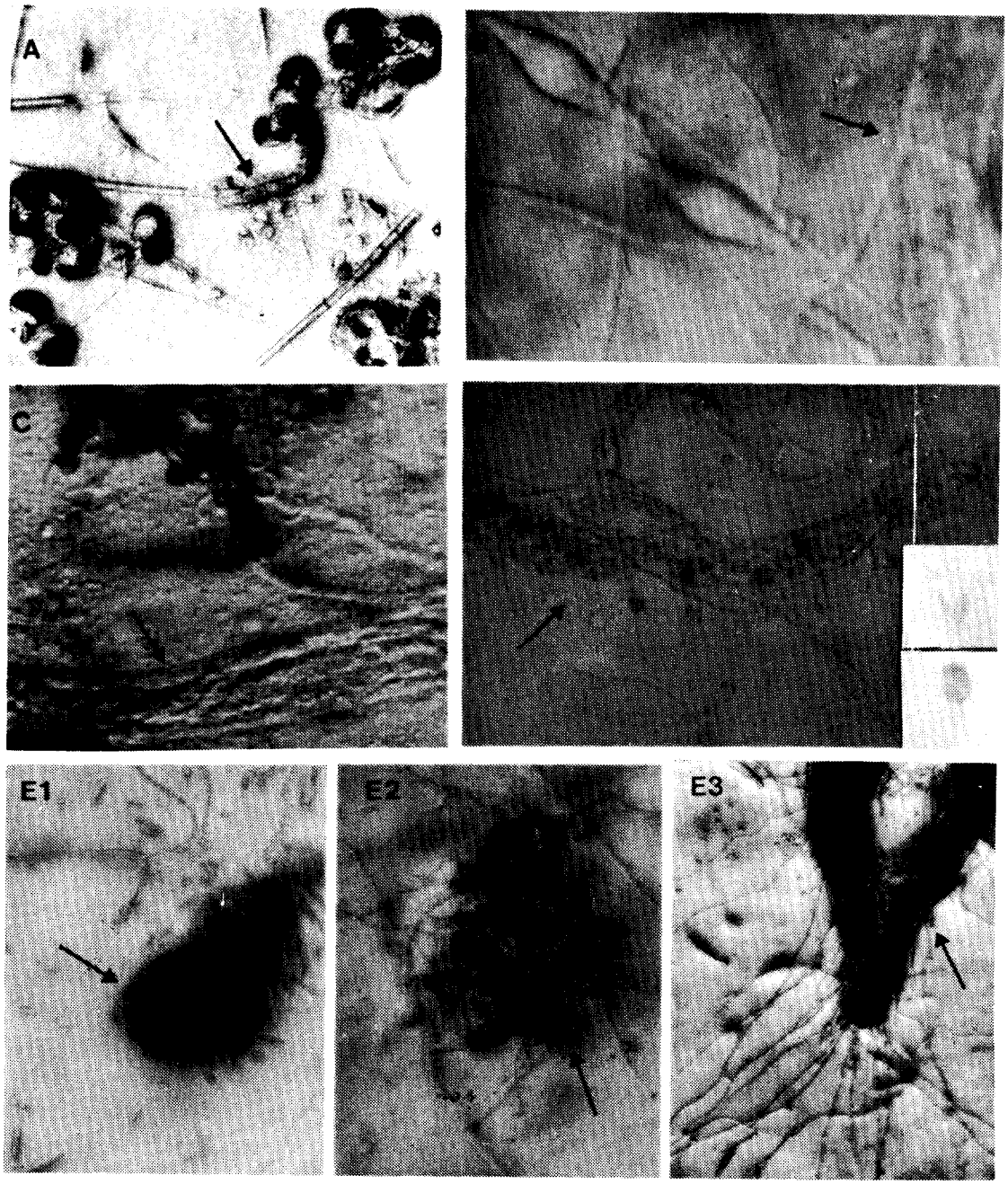

Fig. 1. Illustration of infected nematode(A, B, C, D, E_3) and egg(E_1, E_2) by different nematophagous fungi. *A: Arthrobotrys* sp. *B: Monacrosporium ellipsosporum C: Harposporium anguillulae D: Dactylella lobata* E_1, E_2, E_3: *Fusarium oxysporum*

Table 2. Characteristics of three species of *Arthrobotrys* in pure culture

Species / Characteristics		*A. arthrobotryoides*	*A. conoides*	*A. oligospora*
Colony morphology		white or pale rose spreading rapidly	white to yellow spreading radially	white to yellow spreading radially
Conidiophore	head shape	swollen branched nodular	swollen unbranched nodular	swollen unbranched nodular
	diameter(μm); base	4—5	4—8	5—7
	tip	1.5—3	2—3	2.5—5
Predaceous organ		3-dimensional network	3-dimensional network	3-dimensional network
Groups		Group II	Group IVa	Group IVa
Conidia	shape	ovoid to obovoid, slightly constricted at septum	elongate-obovoid, constricted at septum	elongate-obovoid, constricted at septum
	size(μm)	12.6—32.2×4.2—12.8	18—45×8—15.5	16.5—29.5×8.4—15.5
	number	5—25	5—30	5—20
Sterigmata		wartlike	wartlike	wartlike or short branch-like
Chlamydospore		unkown	hyaline abundant in old culture	yellow present
	shape		round to oblong intercalary, single or in chains	spherical cylindrical intercalary, single or in chains

res, seven nematophagous fungi selected were identified as *A. arthrobotryoides*, *A. conoides*, *A. oligospora*, *D. lobata*, *F. oxysporum*, *M. ellipsosporum*, and *H. anguillulae* which had been known as nematophagous fungi and subjects of a lot of investigations for the control of nematode disease(Stirling & Mankau 1978, Stirling & Mankau 1979, Stirling, Mckenry & Mankau 1979).

We have tried to isolate nematode-trapping fungi as well as endoparasites such as *Harposporium* sp. This fungus was isolated by differential centrifugation method. From the other isolation techniques used in this experiment, however, most of the isolates were aggressive predaceous fungi particularly members of the genera *Arthrobotrys*, *Dactylella*, *Fusarium*, and *Monacrosporium*. This predaceous species might have quickly overrun the plates before the less aggressive or less obvious endoparasites could reveal themselves. Therefore. isolation techniques should be improved so that diverse nematophagous fungi could be isolated.

Influence of some environmental conditions on mycelial growth and sporulation *in vitro*.

Effect of media

Among 14 media tested in this experiment, CMA were the most suitable media for growing all six isolates of nematophagous fungi. Especially slow growing *M. ellipsosporum* grew vigorously only on these two media. The growth of this fungus on other media was slow. Only two fungi such as *F. oxysporum* and *D. lobata* profusely produced conidia on WA. Therefore, it was possible to rule out the fungi from many other fungi in this experiment. Mycelial growth of *D. lobata* and *F. oxysporum* were fairly good on

Table 3. Growth and sporulation of six species of nematophagous fungi on various agar media

Media[a]	Dry Wt(mg) after 14days						Radial growth(cm) after 4 days						No. of spore(×10⁴) after 4 days					
	A_3	A_4	A_5	D_1	F	M_4	A_3	A_4	A_5	D_1	F	M_4	A_3	A_4	A_5	D_1	F	M_4[b]
ANM	5.1	6.1	4.0	56.9	24.4	5.7	6.9	6.3	5.9	5.5	5.4	1.3	1.0	0.1	0.1	826.3	160.8	0.1
BA	0.8	1.3	0.6	42.8	2.7	1.1	5.4	5.8	6.5	3.9	4.8	1.5	—	—	0.5	12.5	53.0	0.1
CMA	10.4	33.4	12.2	41.7	12.7	36.1	7.0	5.3	5.5	6.4	4.9	1.9	2.2	0.8	—	140.0	51.0	0.1
CM	9.4	9.3	9.7	81.9	72.6	10.5	3.5	1.5	3.1	5.4	5.3	0.8	—	—	—	1008.8	44.8	—
CSA	5.7	4.3	6.4	75.2	24.6	4.5	7.6	3.3	5.2	5.8	4.8	1.7	—	—	—	660.0	102.0	0.1
GPA	5.7	11.2	8.5	80.6	22.4	7.9	6.9	6.7	5.6	4.8	5.3	2.4	2.5	—	—	1382.5	116.8	—
LA	2.5	5.8	0.9	44.3	15.6	8.2	7.1	6.5	6.7	5.5	5.5	2.1	0.4	1.4	—	367.5	86.3	0.2
MA	4.9	4.9	2.1	49.4	7.0	4.3	6.5	5.8	5.9	6.1	5.6	2.3	1.0	0.1	—	17.2	14.0	0.1
NA	1.1	2.0	1.1	44.3	5.6	4.2	5.1	4.5	4.5	5.7	5.3	1.7	0.7	—	—	487.5	208.3	0.2
WA	—	0.6	0.6	44.1	0.2	1.1	5.2	5.2	5.9	5.9	5.0	2.3	—	—	—	28.0	17.0	—
OMA	15.7	36.1	14.6	41.8	21.9	34.6	6.0	5.7	6.0	6.1	4.9	2.4	1.5	1.8	1.2	347.5	165.8	0.3
PDA	6.2	6.6	2.5	59.7	16.8	6.3	7.4	6.1	6.4	5.6	5.4	2.2	4.8	0.3	0.9	1437.5	400.0	—
SNA	9.8	26.5	5.3	52.5	43.8	6.8	7.7	5.8	5.8	5.1	5.9	1.9	4.8	1.7	—	266.3	635.0	—
YEA	2.8	10.2	7.3	62.2	13.1	5.3	6.9	5.8	6.0	5.3	4.7	2.0	3.2	4.1	5.5	344.8	415.0	—
LSD .05	0.1	0.1	0.1	0.1	0.1	0.1	0.4	0.4	0.4	0.3	0.3	0.2	0.7	0.5	0.4	124.8	28.4	0.2

[a] ANM, Ashour's Nutrient Medium; BA, Barnes, Agar; CMA, Corn Meal Agar; CM, Crabill's Medium; CSA, Czapek Solution Agar; GPA, Glucose Peptone Agar; LA, Leonian's Agar; MA, Malt Extract Agar; NA, Nutrient Agar; OMA: Oatmeal Agar; PDA, Potato Dextrose Agar; SNA, Sucrose Nitrate Agar; YEA, Yeast Extract Agar

[b] See the footnote of Table 4 for A_3, A_4, A_5, D_1, F, and M_4

all of the media tested. The dry weight of each fungus tested were quite diverse depending on the culture media. *Arthrobotrys* spp. and *M. ellipsosporum* grow best on OM A and CMA. *D. lobata* and *F. oxys porum* grew best on CM. (Table 3).

Nematophagous fungi also differd in sporulation ability on various culture media(Table 3). *A. arthrobotryoides* produced conidia most abundantly on PDA and SNA while *A. conoides* and *A. oligospora* sporulated best on YEA. *D. lobata* was revealed good growth and sporulation on GPA. The mycelial growth of this fungus was somewhat delayed but the conidia production was most suitable on PDA. Although over-all growth of *F. oxysporum* was not satisfactory on SN A, the sporulation was best on this medium.

Table 4. Growth and sporulation of six species of nematophagous fungi grown on Sucrose Nitrate Agar at given temperature ranging form 10 to 30°C at 5°C interval

Temp.	Dry wt(mg) after 14days						Radial growth(cm) after 4 days						No. of spore(×10⁴) after 4 days					
	A_3	A_4	A_5	D_1	F	M_4	A_3	A_4	A_5	D_1	F	M_4	A_3	A_4	A_5	D_1	F	M_4[a]
10°C	3.8	4.3	3.2	4.3	3.7	3.7	—	—	0.5	—	—	—	—	—	—	—	—	—
15°C	3.8	7.5	3.8	12.0	28.7	4.6	1.9	1.8	1.8	0.9	1.9	0.5	—	—	0.5	0.3	3.3	—
20°C	5.5	20.4	22.9	69.5	35.5	5.3	7.9	7.0	6.0	3.3	5.1	1.5	17.5	2.0	—	392.5	158.8	—
25°C	14.8	28.5	10.6	61.5	44.4	6.0	9.3	9.0	8.5	5.3	7.0	2.1	24.8	23.5	8.3	763.8	1185.0	—
30°C	3.5	16.9	7.9	48.6	52.3	4.8	7.2	8.1	6.8	5.2	7.6	2.2	0.5	171.5	0.5	1878.8	915.0	—
LSD .05	0.1	0.1	0.1	0.1	0.1	0.1	0.4	0.3	0.3	0.3	0.3	0.1	2.9	32.2	0.2	117.5	144.2	—

[a] A_3, *Arthrobotrys arthrobotryoides:* A_4, *A. conoides:* A_5, *A. oligospora:* D_1, *Dactylella lobata:* F, *Fusarium oxysporum:* M_4 *Monacrosporium ellipsosporum*

Table 5. Growth and sporulation of six species of nematophagous fungi grown on Sucrose Nitrate Agar at given hydrogen-ion concentration ranging from 3 to 9

	Dry Wt(mg) after 14 days						Radial growth(cm) after 4 days						No. of spore($\times 10^4$) after 4 days					
pH[a]	A_3	A_4	A_5	D_1	F	M_4	A_3	A_4	A_5	D_1	F	M_4	A_3	A_4	A_5	D_1	F	M_4[b]
3	26.1	32.9	17.7	48.1	77.7	5.6	2.7	0.7	0.8	3.9	4.5	1.7	—	—	—	2586	1337	1.0
4	27.1	51.9	32.0	56.3	103.2	8.6	4.3	2.7	2.8	4.1	5.9	2.6	0.3	1.5	0.3	2377	1940	0.5
5	28.2	53.0	43.9	54.4	104.5	9.2	5.7	2.7	4.3	4.1	6.5	2.7	0.3	2.0	—	6170	2287	1.8
6	38.3	57.3	50.6	64.2	106.4	9.0	6.2	3.8	5.5	4.0	6.5	2.7	0.5	5.3	0.3	9522	1822	1.0
7	44.9	47.9	48.9	71.3	50.2	9.1	6.4	5.5	6.1	4.0	7.1	1.1	0.8	6.8	0.5	4087	1262	0.3
8	41.0	34.1	24.9	62.7	87.0	8.2	7.1	5.1	5.6	4.1	6.2	—	3.5	1.8	—	612	1115	—
9	20.8	27.7	28.6	33.9	81.6	10.4	7.0	5.2	5.1	3.6	6.1	—	6.5	0.3	—	341	910	—
L S D.05	0.1	0.1	0.1	0.1	0.1	0.1	0.4	0.2	0.3	0.1	0.4	0.1	4.5	2.5	0.1	85	447	1.4

[a] 1/10M Sodium phosphate, dibasic or 1/5M Citric Acid

[b] See footnote of Table 4 for A_3, A_4, A_5, D_1, F, and M_4

From this results it could be assumed that the best medium for mycelial growth and sporulation of nematophagous fungi was not necessarily coincident.

Effect of temperature

Fungi grew profusely at temperature ranges of 20—30°C(Table 4). *A. oligospora* and *D. lobata* revealed maximum dry weight of mycelium at 20°C and *F. oxysporum* was 30 °C. Generally, radial growth was optimum at 25°C for most fungi. *A. arthrobotryoides, A. oligospora* and *F. oxysporum* produced largest amount of spore at 25°C but *A. conoides* and *D. lobata* were drastically increased at 30°C. At 25°C, both dry weight and sporulation was best for *A. arthrobotryoides. F. oxysporum,* required rather high temperature of 30°C for growth than for sporulation. The other fungi was *vice versa* in temperature requirement for growth and sporulation.

Effect of pH

The optimum pH for mycelial growth of *A. arthrobotryoides* and *A. oligospora* were pH7 and 6 respectively(Table 5). Between pH 6 to 8 was the optimum range for *D. lobata* while pH 4 to 6 was optimum range for *A. conoides* and *F. oxysporum. M. ellipsosporum* grew most slowly at very wide ranges from pH 3 to 9. Generally the radial growth of the fungi was maximized at pH 7 except *A. arthrobotryoides* and *M. ellipsosporum;* this fungus was adapted to acidic condition whereas that one was to alkalic condition.

Sporulation was most abundant for *D. lobata* and *F. oxysporum* within given pH ranges about 1,000—2,000times more than other fungi(Table 5). These two fungi were exclusively promising for their aggressiveness at pH ranges of most natural field soils throughout Korea. Therefore, above fungi were given special interest for their wide adaptability to ecological niches in natural field condition.

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